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Rapid testing for antibacterial susceptibility

1. Existing procedures for testing antibacterial activity are usually based on the inhibition of bacterial growth. More rapid methods might be advantageous.
   a. In dealing with very slow growing microbes, such as Mycobacterium tuberculosis
   b. Where the drug is highly unstable.
   c. When a rapid assay may be vital, in clinical or in biochemical investigations.

2. Other synthetic processes, besides overall growth, are susceptible of measurement, e.g., virus synthesis or adaptive enzyme formation. The latter might be more generally applicable. These methods presuppose that the test organism has already been isolated and grown; they should be sensitive enough to pick up the action of a single colony ± 1 mm diameter.

3. Adaptive enzymes. Speaking generally, bacteria are capable of forming a wider variety and amount of specific enzymes than they mannifest in any singe environment. The capacity of an organism to respond to the chemical stimulus of a given substrate by forming corresponding enzymes is called "enzymatic adaptation". (This process should not be confused with population shifts; adaptive enzymes are physiological responses which are evolved in every competent cell, and which disappear when the stimulus is removed.) As this involves new protein synthesis it is inhibited by <u>almost</u> any reagent which inhibits growth.

4. Measurement of adaptive (induced) enzymes. For practical application of this method, it is essential to have simple, sensitive assays for the enzyme. Fortunately, "chromogenic substrates" have been devised, and more can be contrived, for a number of enzymes which may be expected to be adaptive in, e.g., mycobacteria. As an example for illustration, "ONPG" is such a substrate for lactose (1). ONPG is o-nitrophenyl β-d-galactoside, and is colorless in aquous solution. It is enzymatically split into galactose + o-nitrophenol, which has a deep yellow color at neutral or alkaline pH. It is sensitive enough to detect the enzyme content of a small E. coli colony within a short time.

5. Tentative protocol -- application to drug sensitivity test in mycobacteria. (Assume that lactase is an adaptive enzyme here). Cultures would first have to be grown, either as mass cultures or isolated colonies, on a good growth medium (lacking lactose). Tubes would be prepared of a good growth medium + lactose + ONPG ± inhibitor. They would be inoculated with ca. equal amounts of bacterial mass. If the method is successful, a positive color reaction should appear within a few hours in the control tube. If the organism is resistant to the drug it should also give a positive reaction in the presence of the drug; if it is susceptable, or if the culture is mixed, the reaction will be more or less inhibited. The reaction can, of course, be quantitated by colorimetry.

6. Necessary research. a. Enzymes, technically suitable, which are adaptive. b. Conditions for optimum sensitivity of assay. c. Conditions for optimum effect of drugs. d. Development of new chromogenic substrates if necessary. e. Correlation of findings by this method, and from other laboratory and from clinical observations.

7. Background information. Beinheim (2) has reported enzymatic adaptation for the oxidation of benzoic acid by the BCG strain. The adaptation is quite rapid (can be scored in one to two hours) and is markedly inhibited by streptomycyn and other compounds. However, the assay of this enzyme is cumbersome and depends on Warburg manometric measurements. Other rapid methods have been reviewed (3, 4).

References

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3. Mitchell, R. B. Military Medicine 116

4. Morse, M. L. and Weaver, R. H. 1950 Rapid microtechniques for identification of cultures. Amer. Jour. Path. 20: 481-484.

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